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13. ABSTRACT *(Maximum 200 words)*

Bacterial genes and enzymes encoding the biochemical pathways of anaerobic benzoate and 4-hydroxybenzoate degradation were identified and characterized. These pathways are important because their operation is essential for the complete degradation of many toxic compounds of environmental concern. Also, several of the enzymes of the pathways catalyze novel reactions that may be representative of general biochemical strategies for anaerobic attack on benzene rings. We sequenced a cluster of twenty-four genes from the bacterium *Rhodopseudomonas palustris*. These include twelve genes likely to be involved in anaerobic benzoate degradation and additional genes that convert the related compound 4-hydroxybenzoate to benzoyl-coenzyme A. Physiological data and DNA sequence analyses indicate that the benzoate pathway consists of unusual enzymes for ring reduction and ring cleavage interposed among enzymes homologous to those catalyzing fatty acid degradation.

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MOLECULAR BIOLOGY OF ANAEROBIC AROMATIC BIODEGRADATION

FINAL REPORT

CAROLINE S. HARWOOD

JUNE 3, 1998

U. S. ARMY RESEARCH OFFICE

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UNIVERSITY OF IOWA

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A. STATEMENT OF THE PROBLEM STUDIED.

Many of the toxic wastes that are released into the environment are aromatic compounds. Examples include nitroaromatics, such as TNT, chlorinated aromatics, such as chlorobenzene, and aromatic hydrocarbons like toluene and xylene. Under anaerobic conditions the aromatic carboxylic acids, benzoate and 4-hydroxybenzoate, are formed as key intermediates during the biodegradation of aromatic pollutants. The acids then enter central pathways of anaerobic benzene ring reduction and fission, leading to complete mineralization. Not a single catabolic pathway for the anaerobic degradation of any aromatic compound has yet been elucidated in detail, and at the beginning of this project period practically no work had been done on the genes encoding pathway enzymes. If the potential of bacteria to degrade benzene rings under anaerobic conditions is to be manipulated to realize their full detoxification potential or to produce intermediary compounds that may have commercial value, it will be necessary to understand in detail the metabolic mechanisms involved, to know how the degradation pathways are regulated, and to develop approaches for modifying the genes encoding key enzymes of anaerobic benzene ring degradation.

As an approach to achieving these goals we studied the anaerobic degradation of two selected aromatic acids - benzoate and 4-hydroxybenzoate - by one bacterial species - *Rhodopseudomonas palustris*. Our emphasis was on exploring the genetic basis of aromatic acid degradation. We expect that many of our conclusions will extend to other bacteria and related compounds.

B. SUMMARY OF THE MOST IMPORTANT RESULTS

The major accomplishment for the last project period was that we cloned, sequenced and started to characterize a 25 kb segment of DNA from *Rhodopseudomonas palustris* that includes twelve genes likely to be involved in anaerobic benzoate degradation and additional genes that convert the related compound 4-hydroxybenzoate (4-HBA) to benzoyl-CoA. Work on the genes and on the enzymes they encode led us to propose a probable pathway for anaerobic benzoate degradation. Genes encoding two new enzymes that catalyze two difficult reduction reactions involved in benzoate and 4-HBA degradation were identified by directed mutagenesis and physiological analysis. One of these, benzoyl-CoA reductase, is a novel enzyme that catalyzes benzene ring reduction - the rate limiting step in anaerobic aromatic degradation. The other enzyme, 4-hydroxybenzoyl-CoA (4-OHbenzoyl-CoA) reductase, catalyzes the reductive dehydroxylation of 4-OHbenzoyl-CoA to form benzoyl-CoA. Reductive dehydroxylases are likely to be critical for the biodegradation of plant-derived aromatics in anaerobic environments because these compounds are often hydroxylated. The nucleotide sequences of the genes encoding these enzymes have provided us with significant new insights about probable mechanisms responsible for two demanding reduction reactions. We also completed a study of benzoate-CoA ligases in *R. palustris* and determined that benzoate-grown cells actually have three enzymes that can catalyze the addition of coenzyme A to benzoate. Finally, we purified and characterized 2-ketocyclohexanecarboxyl-CoA hydrolase, the enzyme that is responsible for the ring cleavage step of anaerobic benzoate degradation.

C. LIST OF PUBLICATIONS

Gibson, J., and C. S. Harwood. 1995. Degradation of aromatic compounds by non-sulfur purple bacteria. p. 991-1003. In: R. E. Blankenship et al. (eds), Anoxygenic Photosynthetic Bacteria. Kluwer Academic Publishers.

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D. SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT AND DEGREES AWARDED.

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E. REPORT OF INVENTIONS:

None